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BIOLOGICAL BULLETIN

SOME CONSIDERATIONS CONCERNING THE NATURE AND ORIGIN OF PHYSIOLOGICAL GRADIENTS.

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AUTHOR'S ABSTRACT.

The paper is a survey of the evidence bearing upon certain aspects of the problem of integration or pattern in organisms. The organism represents a definite pattern or integration of some kind. The pattern of the organism is of a higher order of magnitude than protoplasmic pattern since it involves masses of protoplasm or even cells each of which may possess the entire protoplasmic or cell pattern. Protoplasm apart from environment does not appear to possess any inherent mechanism for originating such pattern.

While specific differences appear in the development and differentiation of pattern in each organism, in its more general features such as polarity and symmetry, this pattern appears to be largely independent of specific differences in protoplasmic constitution. This fact suggests that it represents primarily a non-specific or quantitative condition or relation in a specific protoplasm.

Many different lines of evidence which are briefly reviewed indicate, first, that the simplest form of axiate pattern is primarily a gradient in physiological condition, involving the fundamental metabolic reactions as well as various other factors, and second, that such gradients arise in the final analysis from differential exposure to external factors which affect the rate of protoplasmic activity. The primary physiological relations established in this way are those of excitation and transmission but from the first moment of differentiation chemical or transportative correlation becomes possible and plays an increasingly important rôle in the progress of development.

The living organism is manifestly not simply a fortuitous aggregation of substances but represents an order or unity, an integration of some sort, and the problem of the nature of this order and unity, the problem of organismic integration¹ is one of

¹ In view of the fact that the word "organism" which implies the existence of a unity and order in the entity so designated is universally accepted and employed, the word "organismic" is not only biologically and etymologically justified, but fills a need which is becoming more and more apparent.

the fundamental problems of biology. The organism consists in each particular case of a certain material, a protoplasm possessing a specific inherited constitution and it represents a certain pattern which is in some way established in this material, and the problem of organismic integration includes both the problem of pattern and the problem of material. Pattern in the sense in which the word is used here is not merely nor primarily the visible morphological order and arrangement of parts or organs, but rather the underlying physiological order and relation of which the visible morphological order is one result, and the problem of pattern is the problem of the nature of this order. But since organismic pattern always appears in the material which we call protoplasm it is evident that the problem of material, *i.e.*, the problem of the physico-chemical constitution of each particular protoplasm in which the pattern appears is also of fundamental importance. Obviously organismic pattern must be of a kind which is possible in the protoplasm in which it appears and to this extent pattern must depend upon and be determined by the nature of the material. Moreover, protoplasm itself possesses a pattern, that is to say the problem of material resolves itself again into problems of pattern and material, the material in this case consisting of the various constituents of protoplasm, colloid particles, lipoids, electrolytes, water, etc., and the pattern of the order and the relation of these constituents. Similarly for each constituent of a protoplasm the two problems exist and so on until we attain the fundamental conceptions underlying physics and chemistry.

At present, however, we are primarily concerned with the question of the organismic order or pattern as it appears in the protoplasmic material and we must accept protoplasm with its physico-chemical constitution and pattern as given, and attempt to determine how the organism differs from and arises out of protoplasm.

Organismic pattern appears in general to be of a different order of magnitude from protoplasmic pattern. It involves the appearance of orderly differences in and relations between regions or masses of protoplasm or cells each of which may possess the entire protoplasmic pattern. This fact alone presents

great difficulties to any preformistic conception of organismic pattern, for protoplasmic pattern alone does not appear to afford any basis for the origin of this pattern of a much higher order of magnitude. Apparently organismic pattern is in some way superimposed on protoplasm and unless we are content to accept the "vitalistic" conclusions as a solution of the problem, we are forced to believe that in the final analysis organismic pattern arises in some way through the relations between protoplasm and the external world.

Second, organismic pattern as regards its more general features is apparently to a large extent independent of the specific differences in constitution of the different protoplasms. This independence is clearly indicated by the fact that only three types of spatial order appear in organismic pattern. These three are the surface-interior, or centered pattern in which the order is spatially referable to a point, the axiate pattern, in which the order is referable to a line, the axis, and the bilateral pattern, in which the order is referable to a plane passed through the line representing the axis. The spatial plan or pattern of all organisms represents either some one of these patterns or some modification or combination of one or more of them. If organismic pattern were inherent in protoplasm and therefore determined entirely by its specific hereditary constitution we should expect a far greater diversity in the general spatial order in organisms. The diversity appears, however, not in the general pattern but in the character and course of differentiation of its parts, and the degree and character of physiological relation between them. The liverwort *Marchantia* and the flatworm *Planaria* are both axiate and bilaterally symmetrical, *i.e.*, the general spatial pattern is essentially identical in both, but they are very different organisms because the materials, the protoplasms, in which the pattern exists are very different in constitution. Likewise different species of liverwort and different species of planarians differ in definite and characteristic ways because, even though the general features of the liverwort pattern or the planarian pattern are the same in all, the pattern is so to speak worked out or developed in specifically different protoplasms. And finally, both in individual development and in evolution we find in general a

progressive complication in the details of organismic pattern, even though the same general features may exist in widely different stages of development and in widely different organisms, and the course of this complication differs both in the development of different species and in different lines of evolution.

These facts as well as many others suggest that the primary features of organismic pattern consist in a non-specific or quantitative order arising in some way in a protoplasm of specific constitution. We must then, in attacking the problem of organismic pattern, attempt first of all to determine whether there is any evidence for the existence of such a quantitative order as the basis of organismic pattern, and, if we find such evidence, how such an order originates in protoplasm. If the conclusion stated above is correct, viz., that organismic pattern in the final analysis must be determined, not by protoplasm alone, but by the relations between protoplasm and its environment, the problem of the origin of organismic pattern is the problem of determining what particular relations give rise to the primary features of this pattern and how. Two questions then are before us: what is the nature of organismic pattern in its simplest or most primitive stages or forms, and how does such pattern originate?

THE SIMPLEST STAGES OF ORGANISMIC PATTERN.

Surface-interior Pattern.—The cell is primarily an organism, though it may be integrated with other cells to form a part of a multicellular organism. While we have no positive knowledge concerning the origin of cell pattern, the structure of the cell in general suggests that it is primarily what we may call a surface-interior pattern. If this is true the differentiation of nucleus and cytoplasm from the primitive protoplasm possessing in some degree the functions of both, must have resulted in the first instance from differences and relations between surface and interior. Some organisms appear to be even simpler in pattern than the ordinary cell, but most of them are so minute and show so little differentiation of parts that our knowledge of their pattern is very fragmentary. Even in the simplest organisms, however, we should expect to find at least a surface-interior pattern. In the absence of any positive data, discussion of the origin of cell

pattern and of still simpler patterns is of value only in the light of what we can learn concerning other organismic patterns and is therefore postponed (see pp. 176-178).

The surface-interior pattern is primarily a completely radial, spherically symmetrical, or centered pattern and, assuming that organismic pattern arises through relation between protoplasm and environment, this pattern represents such relation in its most general and primitive form. While some cells and some still simpler organisms may possess this pattern alone it is certain that most organisms including all multicellular forms, at least many unicellular forms and probably some of those simpler than cells, possess not only a surface-interior pattern but also an axiate or polar pattern and primarily either a radial or bilateral symmetry with asymmetric modifications in various cases. Both the course of organismic evolution from very early stages, as well as the development of the individual, are based upon this axiate pattern. I have already pointed out (pp. 149-150) that the general similarities of organismic pattern in different protoplasms suggest that such pattern is, at least as regards its fundamental features, in large measure independent of the differences in the specific constitution of different protoplasms and therefore essentially quantitative, *i.e.*, it apparently represents a quantitative relation in a specific protoplasm. It now becomes necessary to examine the data of observation and experiment with reference to the question of the nature of the axiate pattern.

Axiate Pattern.—Many different lines of evidence agree in indicating that axiate pattern in its simplest form is essentially quantitative in character and consists in graded differences in the rate of the fundamental dynamic activities of protoplasm and in the conditions associated with these activities. These graded differences in physiological state have been called axial gradients because so far as the evidence goes they are the primary indications of the existence of an axiate pattern (Child, '15c). They have also been called metabolic gradients, not because it is assumed that they are purely or primarily metabolic in character, but because our knowledge of protoplasm in general indicates that metabolism and more specifically oxidation is a fundamental factor in life and the chief source of the energy of living organ-

isms and because the data of observation and experiment indicate that differences in the rate of metabolism and particularly of oxidation are characteristic features of these gradients. Protoplasm is a system in which the chemical reactions of metabolism are so intimately associated with other factors, *e.g.*, colloid dispersion, active mass of enzymes, permeability of limiting surfaces, electrolyte and water content, etc., that to attempt to distinguish one particular factor rather than another as primary is at present impossible. The axial gradients are not simply oxidative or metabolic gradients but gradients in general physiological state of the particular protoplasm concerned, and in this physiological state oxidation and associated with it other metabolic reactions are important factors. The term "metabolic gradient" as used in this connection means only that the metabolic factor is a characteristic feature of the gradients. The term "physiological gradient" which avoids all specific implications might be substituted for it.

In the development and differentiation of the axiate pattern the apical region or head of the organism arises from the region of greatest activity or highest metabolic or oxidative rate—the "high end" of the major or polar gradient—and other organs at different levels of the gradient. The simplest forms of radial symmetry in which there is no differentiation between different radii are in reality nothing more than an apico-basal pattern and only an apico-basal gradient is present. In the more complex forms of radial symmetry a number of radial axes or gradients arise and the radially arranged parts differentiate in relation to these. In bilateral patterns there is not only a gradient along the longitudinal or polar axis, but bilateral gradients are also present. In most bilateral invertebrates the high region of these bilateral gradients is apparently represented by the median ventral region and in the vertebrates by the median dorsal region. The evidence indicates that the chief axial gradients appear primarily in the superficial regions of the cell or multicellular body and in many of the simpler organisms, *e.g.*, many protozoa (Child, '14*b*) and plant cells they are throughout life present only or chiefly in the superficial regions, but where definite localized internal organs with an axiate pattern exist, these also show

axial gradients in all cases examined, though these organ gradients do not necessarily coincide in direction with the primary gradients. As was pointed out nearly five years ago (Child, '15c, pp. 54, 60) the primary gradient or gradients may persist throughout life, but do not necessarily do so. They may undergo modification and complication in various ways during the course of development: the originally quantitative relations may become qualitative, new gradients may arise in certain parts or organs, in some cases preëxisting gradients may be broken up, obliterated or reversed, one kind of axiate pattern may be replaced by another, and so on. In all cases, however, the sequence of events is definite and orderly and many features of it can already be interpreted in physiological terms, and usually the pattern even in the fully developed organism shows a very definite relation to the primary gradients. The evidence for the existence of metabolic or physiological axial gradients is varied and extensive and only a brief summary is possible here.

Structural and Developmental Gradients.—First of all many eggs and embryos show an apico-basal gradient in protoplasmic structure and content, *e.g.*, the gradient in yolk accumulation in many animal eggs and embryos and the gradient in protoplasmic density and vacuolation in many plant embryos (Child, '15c, Figs. 8, 19) and in the vegetative axes of many of the simpler plants.

A gradient in the rate of cell division, growth and differentiation in relation first of all to the primary or apico-basal axis, later in relation to other axes, is a very general feature of at least the earlier stages of development. This gradient appears first in the rate of cell division and size of cells along the apico-basal axis in a large proportion of both animal and plant eggs (Child, '15c, Figs. 10, 11, 18, 19) and in many plant axes, as a gradient in rate of division and cell size from the growing tip basipetally (Child, '15c, Figs. 20, 21, 22, 36-39). It also appears in the progress of morphogenesis and differentiation along the axes particularly in animals, although in plants, except for the fact that the growing tip itself remains embryonic, the course of differentiation is also in general basipetal. Moreover, in bilateral animals and plants a similar bilateral developmental gradient ap-

pears more or less clearly. In at least most bilateral invertebrates differentiation of the body-wall and its organs progresses from the median ventral region laterally and dorsally (Child, '15c, Fig. 13) and in vertebrates from the median dorsal region laterally and ventrally, as a moment's examination of the chick embryo shows. The so-called law of antero-posterior development is merely a recognition of the existence of this developmental gradient in the longitudinal axis, and it is, moreover, only a partial statement of what might be called the law of axiate development. We also find evidences of the existence of a polar axial gradient in the rate of regulatory development and the position and proportions of organs in isolated pieces from different levels of the body in various forms (Child, '07b, '07c, '11b; Hyman, '16).

The Evidence from Susceptibility.—The existence of the axial gradients is perhaps most readily demonstrated through the susceptibility of organisms to the action of various external agents. It has been determined experimentally for many species of animals including all the chief phyla and many of the smaller groups and nearly a hundred species of algæ among plants that axial gradients in susceptibility to the action of at least a wide range of external agents exists. The agents used in these experiments include cyanides, many anesthetics such as alcohol, ether, chloroform, chloretone, some of the urethanes, etc., carbon dioxide and various acids, alkalies, neutral salts, certain alkaloids, vital dyes and physical conditions such as extremes of temperature and the negative factor lack of oxygen.¹ Potassium cyanide has been much used in these experiments first because very low concentrations are effective and second, because it has been found by a large number of investigators to be a powerful inhibitor of protoplasmic oxidation, and susceptibility to cyanide may there-

¹ The data on susceptibility as determined by death and disintegration of cells or tissues, so far as they have been published, appear in the following papers: algæ, Child, '16c, e, '17a, b, '20a, protozoa, Child, '14b, Hyman, '17; celenterates, Child, '18, '19b, Child and Hyman, '19; ctenophore, Child, '17c; Planaria, Child, '12, '13a, '13b, '14c, d, '16b, '19c, d; echinoderm eggs and embryos, Child, '15a, '16a; annelids, Child, '17d, Hyman, '16; amphibia, Bellamy, '19; miscellaneous, Child, '14a, '15c, pp. 50-62. Further data on susceptibility gradients in protozoa, ctenophores, hydrozoa, flatworms, echinoderms, annelids, fishes and amphibia obtained by Bellamy, J. W. MacArthur, Hyman and Child are not yet published.

fore be regarded as to some extent a comparative measure or indicator of rate of oxidation in the parts, organs or individuals of a particular species.¹

Comparison of the data on susceptibility with data obtained by other methods has shown that a general non-specific relation between susceptibility and physiological condition exists, at least in the simple organisms and early developmental stages, where the differentiation of organs is not so far advanced that a more or less specific action of particular agents or particular organs is involved. This relation is as follows: to a certain range of concentrations or intensities of the agents used, which is experimentally determined to be above the limit of acclimation or tolerance for the particular species examined, susceptibility varies

¹ For the literature on the action of cyanide see Hyman, '19. Allen, '19a, and Hyman, '19, have shown that KNC greatly decreases oxygen consumption in *Planaria*. I have found that KNC decreases CO₂ production in *Planaria*, though apparently less than it decreases oxygen consumption and that KNC and lack of oxygen are to some extent additive in their action on CO₂ production (Child, '19c). Lund claims that KNC does not decrease oxygen consumption in *Paramæcium* (Lund, '18), but it may be pointed out that the concentrations of KNC in his experiments give a solution of very high alkalinity which produces extreme stimulation with intense motor activity often continuing for several hours until the inhibiting action of the cyanide appears. With cyanide solutions brought almost or quite to neutrality there is no apparent stimulation and no increase in motor activity. Lund did not neutralize his KNC solutions, therefore the total oxygen consumption undoubtedly does not represent the action of cyanide alone, but includes the stimulating effect of the alkali. Repetition of the experiments in cyanide solutions with H ion concentration approximately that of the normal environment of *Paramæcium* is of course necessary before final conclusions are possible, and a repetition will be undertaken in this laboratory as soon as opportunity permits.

More recently Lund ('20) has shown that KNC and lack of oxygen do not act in the same way upon *Planaria* since with lack of oxygen CO₂ production is not decreased during the period of the experiment, while it is decreased by KNC. The method of CO₂ determination used by Lund is, as he has shown (Lund, '19), not very accurate, but leaving out of account the possibility of technical error, the work of various investigators indicates very clearly that the effect of cyanides on protoplasm is not identical with the effect of lack of oxygen. I have pointed out that cyanide and lack of oxygen are to some extent additive in their action and that their action must therefore be similar or identical in certain respects. Up to the present, therefore, Lund's work affords no grounds for modifying the conclusions reached by earlier investigators concerning the action of cyanide, and we are still justified in believing that susceptibility to cyanides is in a general way and to some extent a measure or an indicator of rate of oxidation.

directly with, though not necessarily proportional to the general physiological activity of the protoplasm. To a certain lower range of concentrations or intensities, also experimentally determined for each species, the rate and degree of acclimation or acquirement of tolerance vary directly, though not necessarily proportionally, to the general physiological activity of the protoplasm, and the rate and degree of recovery after temporary exposure to the action of the agent also vary in the same way. The metabolic reactions and particularly the oxidations are important factors in the general physiological activity of protoplasm and many lines of evidence show that susceptibility may be used within certain limits and with certain precautions as a rough comparative measure or indicator of the rate of oxidation. This of course does not mean that all the agents used in determining susceptibility act directly on the oxidations. Undoubtedly different agents act in very different ways upon living protoplasm but the general non-specific character of the susceptibility gradients indicates the interrelation of different processes and conditions in the protoplasmic system. The difference between a region of high and one of low rate of oxidation unquestionably involves not simply the oxidative reactions but many other factors, *e.g.*, colloid dispersion, permeability of limiting surfaces, active mass of enzymes, etc., and within physiological limits change in the physiological state involves changes in all these factors. Apparently the general relation between susceptibility and physiological state is primarily an expression of the fact that the dynamic equilibrium of the more active state is, on the one hand, more readily upset by any extreme external action than that of the less active state and, on the other, undergoes more rapid and more complete recovery from temporary alteration. In short, so far as susceptibility is non-specific and quantitative, it is apparently an indicator of the quantitative aspects of physiological state in protoplasm. Susceptibility to the higher, directly lethal concentrations and intensities can be determined by survival time and in many cases loss of motor activity, swelling or shrinkage of cells or other changes preceding death can be used as a check on survival time. To lower concentrations, which are not directly lethal, susceptibility can be determined by

the degree of inhibition, acclimation or recovery in growth, development, motor activity, etc., in different body-regions or individuals.

The data on susceptibility have demonstrated the existence in a large number of organisms of definite susceptibility gradients as characteristic and as the earliest distinguishable features of axiate pattern, not only for the organism as a whole, but for various axiate organs and parts. In all cases the differences in susceptibility correspond to differences in structure, rate of growth, development and differentiation, and in certain cases it has been possible to show that susceptibility gradients correspond to gradients in oxygen consumption and carbon dioxide production.

In various cases it has been possible to follow the apico-basal or antero-posterior gradient from the egg and to show the modifications which occur during the course of development, and the appearance of new gradients in particular organs, or in connection with agamic reproductive processes. In the simpler organisms the susceptibility gradients are wholly or largely superficial. In the ciliate infusoria, for example, axiation and morphological differentiation are, at least to a very large extent, confined to the ectoplasm, and in the forms which have been examined the ectoplasm alone shows a gradient in susceptibility (Child, '14*b*). As regards many plant cells the same is also true. In many of the monosiphonous algæ with elongated cells the apico-basal susceptibility gradient is very distinct within the limits of a single cell (Child, '16*c*, *e*, '17*a*, *b*, '20*a*) as well as in the axis as a whole. Modifications with advancing age, cessation of growth, budding, etc., appear very clearly in the changes in susceptibility.

In some animals the primary gradients persist throughout life, while in others the original gradients may completely disappear during development. In the development of the hydroid, for example, a very distinct gradient appears in the unfertilized egg and cleavage stages, and the planula shows a well marked apico-basal gradient. But as the time of attachment of the planula approaches, this gradient gradually becomes less and less distinct, and the planula attaches itself by the end which was originally apical, a fact which has long been known. After attach-

ment the original gradient disappears completely, a new gradient in the opposite direction arises at the opposite end, and the first hydranth arises from the high end of this new gradient. In other words, the phenomenon which Loeb and others have called heteromorphosis is a normal feature of hydroid development. Again in the polyclad turbellaria the early stages of development show a very distinct antero-posterior susceptibility gradient, the head arising from the most susceptible region, but in the later stages, so far as they have been examined, a reversal in the susceptibility relations occurs, at least in the superficial regions, and in the adult worms the ectoderm of the head is less susceptible than more posterior levels. These data on hydroids and polyclads have not yet been published in full. In the annelids a second gradient in the opposite direction from the primary antero-posterior gradient appears at least superficially in the posterior regions as the result of the development of the posterior growing region (Hyman, '16; Child, '17*d*). But whatever the changes, they are definite and orderly and associated with the course of development in each species.

As development and differentiation progress, the indications of more or less specific relations between particular regions or organs and particular agents become more frequent. In *Planaria dorotocephala*, for example, we find that the lateral margins of the body are more susceptible superficially than the median regions to alkalies, while in neutral and acid agents there is little difference between median and lateral or the median ventral region is slightly more susceptible than dorsal and lateral. With methylene blue of certain concentrations Mr. McArthur has found that susceptibility of the median ventral region is distinctly greater than that of dorsal and lateral regions. The early embryonic stages of *Planaria* have not been available for work on susceptibility, but the data on other flatworms and various other bilateral invertebrates indicate that primarily the susceptibility of the median ventral region is higher than that of dorsal and lateral regions and the outgrowth of tissue from cut surfaces in pieces of adult *Planaria* suggests a greater parenchymal activity in the median ventral region. In the previously published data on the gradients in *Planaria* (Child, '13*b*) it was noted that the

relations as regards median, lateral and dorsal were not entirely clear. As regards the alimentary tract, various facts indicate that in the adult the region of greatest activity and highest susceptibility is in the middle of the body about the base of the pharynx and that a gradient of decreasing activity extends in both directions from this region, but since the alimentary tract is an internal organ and is not readily separable from other parts, it is difficult to obtain conclusive evidence on this point. The antero-posterior gradient of ectoderm and body-wall persists throughout life, but with the appearance of new zooids at the posterior end new gradients arise in that region, or more strictly speaking, the original gradient undergoes modification. Apparently the primary embryonic relations have undergone more or less alteration, even in *Planaria*.

With the appearance of more or less specific relations between particular regions or organs and particular agents, the value of the susceptibility method as a means of distinguishing general quantitative differences and relations is of course greatly decreased. In the higher animals apparent specificity of relation between particular organs and particular agents is much more evident than in lower forms and becomes increasingly complex with the progress of differentiation, but even in these forms the general non-specific susceptibility relations appear in the earlier developmental stages, at least in all forms examined. While caution is always necessary in interpreting the data of susceptibility in non-specific quantitative terms, it has become more and more evident as the data have accumulated that general non-specific susceptibility relations do exist, particularly in the simpler organisms and in the earlier stages of development, and that they are indications of fundamental physiological features of organismic pattern.

It has also been possible to control and modify development in definite predictable ways through the differential susceptibility of different levels of the axial gradients. Such modifications consist in differential inhibition, differential acceleration, differential acclimation and differential recovery, each representing a definite teratological type. In cases of differential inhibition with respect to a gradient the degree of inhibition varies directly with

the susceptibility and activity of different levels. The region of greatest activity is most inhibited and less active regions are less inhibited. In the apico-basal or antero-posterior axis for example, the degree of inhibition is greatest in the apical or head region and decreases basipetally or posteriorly, consequently the positions and proportions of parts are altered in a definite way, the apical or head-region being relatively smaller and the basal or posterior regions relatively larger than in the normal. Microcephaly, for example, is a characteristic result of differential inhibition along the polar axis. In differential acceleration the alterations of proportion are in the opposite direction and mega-cephalic forms result. In differential acclimation and recovery growth or development is first inhibited to some extent, but the more active levels of a gradient acclimate or recover more readily and more completely than the less active, so that in these cases growth or development is finally relatively more rapid or greater in amount apically or anteriorly than basally or posteriorly.

Similar modifications also appear with respect to the symmetry gradients. In differential inhibition in bilateral forms, for example, median regions are more inhibited, while in differential acceleration they are more accelerated than lateral, and in differential acclimation and recovery median regions are finally less inhibited than lateral.

These various modifications are, with respect to their more general features, non-specific as regards agents, and all except the differential accelerations which require the action of accelerating agents can be produced in some degree by a large number, probably by all agents which inhibit general protoplasmic activity and which in lower concentrations or intensities permit at least some degree of acclimation or recovery. Moreover, modifications of the same general type with respect to a particular axial gradient can be produced in widely different organisms, *e.g.*, flatworms, echinoderms, fishes, frogs. In many respects these definite developmental modifications constitute the strongest evidence for the relation between susceptibility and the rate of fundamental activities of living protoplasm.¹ And

¹ Data on the control and modification of development through differential susceptibility have appeared as follows: Child, '11a, '16d, '17d; Bellamy, '19; and further data on hydrozoa, echinoderms and amphibia are still unpublished.

finally, differential susceptibility, as a relation dependent primarily upon quantitative rather than upon specific or qualitative differences in the physiological state or activity of protoplasm, provides a simple and adequate basis for the interpretation of much of the work on experimental teratogeny and many of the teratological forms occurring as "accidents" in nature. The cases of cyclopia and microcephaly in fishes experimentally produced by Stockard ('07, '09, '10, '11, etc.) are essentially similar to the inhibited types of head in *Planaria* (Child, '11a, '15c, pp. 105-117) and to the differential inhibitions in the sea urchin (Child, '16d) and in amphibia (Bellamy, '19, and further data not yet published). All these cases involve a greater degree of inhibition of apical or anterior and median as compared with basal, posterior and lateral regions and all are non-specific in origin, *i.e.*, can be produced by the action of various agents and conditions.

The susceptibility method makes no pretense of being an exact quantitative method of measuring metabolism or oxidation, nor is it to be regarded as taking the place of any other method of investigating physiological condition or rate of metabolism or oxidation. Its chief value is as a supplement to other methods. In the first place it enables us to demonstrate the existence of certain characteristic, non-specific, regional differences in physiological condition in organisms, which because of their unicellular character or their small size, or because of the complications introduced by separating different body regions are not available material for other more direct and more exact methods. Even in these forms, however, the action of external chemical agents is in general from the surface inward, consequently the information given by the susceptibility method concerns first of all the superficial regions of the cell or body, but it is possible in many cases to learn something concerning differential susceptibility of internal parts and organs.

Second, by the modification and control of development through differential susceptibility, the method enables us to show that the differences in condition indicated by differences in susceptibility are fundamental factors in organismic pattern. The conclusions concerning the relation between susceptibility and

rate of metabolism or oxidation mean no more than that rate of metabolism or oxidation is so far as the evidence goes a factor in the conditions which determine susceptibility, but it is not claimed that this conclusion is universally valid. Undoubtedly in the more highly differential organisms and in more advanced stages of development the qualitative differences in different organs may determine differences in susceptibility which are more or less specific as regards both organ and agent, but in the simpler forms and the earlier stages the similarity of the susceptibility gradients in widely different organisms and with a great variety of agents renders their non-specific character sufficiently clear, and many lines of evidence, both direct and indirect, indicate their relation to rate of metabolism or oxidation. The nature and degree of that relation in each particular case and for each particular agent remains of course to be determined by other methods of investigation. The susceptibility method has served to bring to light certain characteristic features of organismic pattern which have not previously been clearly recognized, viz., the gradients, but conclusions concerning the exact nature of these gradients are possible only on the basis of all the different lines of evidence obtainable, and at present of course cannot be final.

Susceptibility in Relation to Permeability.—In many forms, both plants and animals, in which susceptibility gradients exist, corresponding gradients in the rate of penetration of certain substances, particularly the vital dyes, neutral red and methylene blue, which have been most extensively used in these experiments, have also been demonstrated.¹ The existence of these gradients in rate of penetration raises the question whether the susceptibility gradients are not primarily gradients in permeability of the protoplasmic surfaces to the agents used. While there is no doubt that a gradient in permeability is one aspect of the axial gradient, our conception of the relation between permeability and the gradient must depend very largely upon the terms in which we define permeability. If permeability is dependent only on the physical condition of the limiting surface

¹ Much of this work has been done by Mr. J. W. MacArthur and is not yet published. For some observations on algæ see Child, '20a.

and independent of chemical activity, the axial gradients are manifestly something more than mere permeability gradients. But the protoplasmic limiting surface or membrane is certainly polyphasic in constitution, since it is alive and the seat of more or less chemical activity, and its permeability depends upon its living condition and changes when it dies. Moreover, susceptibility as determined by the higher concentrations and intensities of external agents depends rather upon the destruction or alteration of the limiting surface as a living membrane than upon the passage of the agent through the living membrane into the interior of the cell. The susceptibility gradients can be demonstrated not only by agents to which the protoplasmic surface is highly permeable, *e.g.*, vital dyes, various anesthetics, but by those to which it is highly impermeable such as mercury and copper salts, and by extremes of temperature and the negative condition, lack of oxygen, which do not involve the action of any external chemical agent upon the surface.

A brief consideration of the question of permeability in relation to the action of KNC on *Planaria* is of interest here. The susceptibility gradients appear very clearly in KNC: in $m/1000$ KNC, for example, the survival time of the head of *Planaria dorotocephala* is about half or two thirds that of the least susceptible regions of the body-wall. It has been shown, however, that KNC produces a completely reversible decrease of 80-90 per cent. in the oxygen consumption of *Planaria* (Allen, '19a; Hyman, '19), a fact which certainly indicates that the permeability of *Planaria* protoplasm to cyanide is relatively high, and that the differences in permeability in different regions cannot be very great. If the differences in survival time of different body-regions result from differences in rate of penetration of the cyanide, then the rate of penetration in the most susceptible regions must be at least nearly double that in the least susceptible regions, and if this is true, we should expect that in the determinations of oxygen consumption some parts of the body would be dead long before a total decrease of 80-90 per cent. had occurred. In fact, it is apparently not possible to interpret susceptibility to cyanide in *Planaria* solely in terms of rate of penetration.

Moreover, the phenomena of all differential acclimation and recovery in growth and development indicate very clearly that the metabolic activity of protoplasm is a factor in susceptibility. With the advance of our knowledge it becomes increasingly evident that the factors concerned in the permeability of living protoplasm are essentially those concerned in other aspects of life and that permeability is an expression of the physiological state of the plasma membranes. If we admit this, differences in permeability are themselves to some extent indicators of differences in physiological state, but it still remains true that susceptibility is not simply a matter of the rate of penetration of the plasma membranes but rather of the rate of killing or alteration of the membranes and superficial regions of protoplasm by an external agent. Different external agents may and undoubtedly do act chiefly or primarily upon different factors concerned in the maintenance of physiological state, but since these different factors are mutually associated in such maintenance, the general result as regards susceptibility, *i.e.*, the general effect on the physiological state, may be and is the same for at least many different agents. In short, susceptibility is within certain limits and in a general way an index of physiological state in protoplasm and the axial gradients in susceptibility are therefore significant, particularly when their existence is confirmed by other methods, as indicating the existence of non-specific or quantitative differences as the earliest distinguishable features of axiation.

Demonstration of the Gradients by Reduction of KMnO_4 and by the Indophenol Reaction.—The axial gradients have also been demonstrated in many forms as a differential in the rate and amount of reduction of potassium permanganate by protoplasm. It is a well known fact that KMnO_4 is reduced by protoplasm and the reduced salt appears on or in the protoplasm as a brown or blackish precipitate. All axiate forms examined including numerous protozoa, eggs, embryonic and larval stages or adults of the lower invertebrates, echinoderms and smaller arthropods and various algæ among plants show gradients in the rate of staining by permanganate corresponding to the gradients demonstrated by susceptibility and other methods. The precipitation of the reduced salt and the appearance of the brown color appar-

ently begins on the external surface of the protoplasm, often within a few seconds after the organisms are brought into the solution and the differences in rate of precipitation and staining at different levels of an axis are usually very marked. Penetration into the protoplasm occurs rather slowly, its rate depending somewhat on concentration and it is certainly not to any large extent dependent on permeability of living membranes but rather on the killing of the protoplasm from the surface inward.

If the reaction is allowed to continue to completion in excess of permanganate the whole organism may become opaque black and no gradient is visible, but many small organisms thus stained, *e.g.*, blastulæ, hydroid planulæ, small monosiphonous algæ, can be made more or less transparent after such staining by hardening, clearing and mounting, and in such cases the gradient in staining appears. Under these conditions the gradient represents a gradient in the total amount of reduction of permanganate of which the protoplasm is capable and it is highly significant to find that the protoplasm of the apical region of a blastula or an alga axis, for example, is capable of reducing more permanganate than more basal levels. If the organism is first killed by some other agent, *e.g.*, various histological fixing agents, heat, etc., reduction and staining are uniform, or in some cases slight traces of the gradients are still present, but after a few days in alcohol they are completely absent in all cases examined.

Since the chemical reaction concerned here is an oxidation-reduction reaction, the rate and amount of reduction of permanganate must be associated in some way with the oxidative activity of protoplasm, the regions of higher rate of oxidation showing a higher rate and greater total amount of reduction. We find that the permanganate gradients correspond with those demonstrated by other methods, *i.e.*, the more active and more susceptible regions reduce permanganate more rapidly and in larger amount than the less active and less susceptible. This method is a very delicate one and, I believe, of considerable value as a means of determining regional and axial differences in physiological state, particularly in small organisms.

In certain cases the indophenol reaction has been used to demonstrate the existence of axial gradients in blastulæ and

gastrulæ. This reaction is an oxidation which is catalyzed by oxidizing enzymes and which results in the formation of indophenol in the form of a blue precipitate. In living starfish blastulæ and gastrulæ (Child, '15a) a distinct apico-basal color gradient has been observed by this means, the formation of indophenol occurring most rapidly in the cells of the apical region. If the animals are killed by some other agent before the indophenol reaction the blue color is much less marked and the gradient is absent.

Gradients in Electric Potential.—Axial gradients in electric potential have been found by Drs. Bellamy and Hyman to exist in axiate animals so far as examined, the region of greatest physiological activity as indicated by other methods showing in general the highest electro-negativity through the galvanometer. These data are not yet published. Observations along these lines were first made by Mathews ('03) on hydroids, Waller ('03) on many different organisms and organs and Hyde ('04) on various eggs, and both Mathews and Waller point out the probable relation between electric potential and physiological or metabolic activity. Morgan and Dimon ('04) in a study of electric potential in the earthworm found that in general the two ends were electro-negative to middle regions and concluded that the potential differences were not related to physiological polarity. We know now, however, that the earthworm and other annelids develop very early a growing region of high physiological activity at the posterior end (Hyman, '16; Child, '17d) and that the body in later stages shows two gradients in opposite directions. The observations of Morgan and Dimon do not therefore conflict with those of others on other animals. More recently Tashiro ('17 and earlier papers) on the basis of his work on CO₂ production in the nerve fiber has pointed out the probable relations between the electric phenomena and metabolic activity in nerve, and Hyman ('18) has also suggested that bioelectric phenomena in general are primarily due to differences in metabolic activity. While differences in potential undoubtedly may arise in organisms from other causes than differences in metabolism or oxidation rate the facts in general indicate that such differences are very generally associated with differences in metabolic rate. In

any case the axial gradients in potential indicate the existence of graded differences in physiological condition along the axes and in the light of other data, there can be little doubt that differences in rate of metabolism or more particularly oxidation play at least a very important part in determining the electrical gradients.

Oxygen Consumption and Carbon Dioxide Production in Relation to the Gradients.—The direct determination of respiratory activity in different body regions by means of oxygen consumption and CO_2 production requires the separation of the regions concerned and therefore introduces various complicating factors. Only in the simpler organisms is it possible to maintain such separated pieces of the body in anything like a normal condition, and even here the operative procedure involves stimulation and may be followed by depression, and cell division and growth begin at the cut surfaces within a few hours after the section. Moreover, in organisms with localized and differentiated internal organs the axial gradients do not necessarily run in the same direction in all organs and a piece of the body from a particular level may represent a low level of the body wall gradient and a high level as regards certain internal organs, or vice versa. In *Planaria* for example the mouth is near the middle of the body and the activity of the alimentary tract probably decreases from the mouth in both directions, but in the body wall the gradient is from the head posteriorly. If this is true, a piece of the *Planaria* body from levels near the head represents a high level of the body wall gradient and a low level of the alimentary tract, while a piece from near the mouth represents the reverse condition. Unless we can eliminate one or the other of these gradients the oxygen consumption and CO_2 production of such pieces is not likely to give us any very definite information as regards either gradient. In this case, however, we can bring the alimentary tract into a more or less quiescent condition by starvation, and then we find that a gradient in oxygen consumption and CO_2 production does exist in the body wall, the high end being at the head with a second rise in the region of the posterior zooid. The data along this line are as yet mostly unpublished, but one paper on CO_2 production in *Planaria* has already appeared (Robbins and Child, '20) and work on oxygen consumption has been done

by Dr. Hyman with similar results. But the relative amount of alimentary tract tissue in pieces of a given weight taken near the head is less than in pieces taken near the mouth, so that even in animals which have been starved for some time the difference in total oxygen consumption or total CO_2 production of pieces from different regions is not strictly comparable with the regional differences in susceptibility of the body-wall. Where it has been possible thus far to use these methods we have found that the axial gradients demonstrated by other methods are also gradients in oxygen consumption and CO_2 production.¹

Gradients in Organs.—By one method or another or by several different methods, the existence of physiological gradients in various axiate organs or parts of many organisms has been demonstrated, *e.g.*, in various reproductive axes and in the "hairs" of algæ (Child, '16c, e, '17a, '20a), in the larger, slow moving flagellum of *Noctiluca*, in the tentacles of hydrozoa

¹ Allen ('20) has recently reported failure to find differences in CO_2 production corresponding to the antero-posterior gradient in pieces of *Planaria agilis*. This is a different species from the one used in this laboratory, but conditions are probably not fundamentally different in the two. It may be pointed out, however, that the differences in CO_2 production in pieces from different body-levels in *Planaria dorotocephala* are not very great (Robbins and Child, '20) and that Allen's failure to find such differences in *P. agilis* is probably due to the fact that the activity of the alimentary tract was not sufficiently decreased by starvation to permit the differences in the body wall to appear. A study of *P. agilis* is now being made in this laboratory by Dr. Hyman and it has been found that the alimentary tract of this species is considerably larger in relation to the size of the animal than in *P. dorotocephala*. An individual of *P. agilis* of given size is able to take in a much larger quantity of food than a *P. dorotocephala* of the same size. In well fed individuals of *P. agilis* oxygen consumption per unit of weight is only about two thirds that in well fed individuals of *P. dorotocephala* of the same size. Much if not all of this difference is undoubtedly due to the fact that a much larger proportion of the total weight of *P. agilis* than of *P. dorotocephala* consists of food in the alimentary tract or of reserves which are taking little or no part in respiration. Moreover, as might be expected, *P. agilis* starves and undergoes reduction much less rapidly than *P. dorotocephala* and Allen's failure to find an increase in rate of oxygen consumption in the later stages of starvation was due merely to the fact that the starvation period in his experiments was not long enough. Dr. Hyman has found that an increase in oxygen consumption does occur in this species as well as in *P. dorotocephala* and *P. maculata* in the later stages of starvation, but under similar experimental conditions the rise begins considerably later and progresses more slowly in *P. agilis* than in the other species.

(Child and Hyman, '19; Child, '19*b*) in the plate rows of Ctenophores (Child, '17*c*), the growing arms of echinoderm larvæ, branchiæ and sensory tentacles of various annelids, the growing tail of the ascidian and the amphibian tadpole, etc. Dr. Hyman has found that the embryonic heart of the chick and of the fish represents a susceptibility gradient with the high region at the sinus end. But the most extensive work on the metabolic gradient of any organ is that of Alvarez and his assistants on the vertebrate alimentary tract.¹ They have found in the small intestine a gradient in irritability, latent period, tone rhythm, conduction and susceptibility to various drugs and gradients in at least some of these conditions in the wall of the stomach and colon. Tashiro ('17 and earlier papers) has found a gradient in CO₂ production in certain nerves, the direction of functional conduction being down the gradient and in certain of these nerves a susceptibility gradient has been observed (Child, '14*a*).

In an investigation of the respiration of ground nervous tissue C. G. MacArthur and Jones ('17) have found differences in rate of respiration in different parts of the central nervous system which indicate the existence of a gradient in rate of respiration. The rate of respiration is highest in the cerebrum and decreases in the various parts in the following order: cerebellum, midbrain, medulla, corpus callosum, spinal cord, nerve. The authors find that gray matter consumes about twice as much oxygen and produces about one and one half times as much CO₂ as white matter, and some of the differences in respiratory rate in different parts of the nervous system are doubtless due to differences in proportion of white and gray matter. For example, the relatively low rate of the corpus callosum is undoubtedly associated with the fact that it consists of white matter, nerve fibers, rather than cells. But the differences in respiratory rate between cerebrum and cerebellum and between midbrain, medulla and spinal cord are scarcely to be accounted for in this way. These differences constitute highly significant evidence for the existence of an axial gradient in rate of respiration in the central nervous system.

All the evidence is in agreement as regards the existence of

¹ Alvarez, '14, '15*a*, *b*; '16*a*, *b*; '17*a*, *b*; '18*a*, *b*, *c*; Alvarez and Starkweather, '18*a*, *b*, *c*, '19; Alvarez and Taylor, '17*a*, *b*; Taylor and Alvarez, '17.

these physiological axial gradients. Thus far every living physiological axis examined has given evidence of the existence, at least in the earlier developmental stages of such a gradient, and in many cases the experimental methods show the presence of a gradient where structural, or other directly visible indications of its presence are absent. In whatever terms we may finally interpret these gradients, there can be no doubt concerning their existence. They are physiological facts, and their significance for localization, differentiation and functional relation is already demonstrated.

THE ORIGIN OF THE AXIATE PATTERN IN NATURE AND EXPERIMENT.

It is a familiar fact that physiological axes persist through certain agamic reproductive processes and are therefore inherited by the individuals resulting from such reproduction, but in at least many eggs the axiate pattern apparently arises *de novo* during the growth of the egg. Granting that axiate pattern in its simplest form is a gradient pattern as already pointed out, we have at present no grounds for believing that such pattern is inherent in protoplasm, or that it can arise *de novo* in protoplasm apart from the action of environmental factors. Apparently a differential exposure of the protoplasm to some environmental factor or factors which affect its rate of activity, *i.e.*, which are primarily quantitative rather than specific or qualitative in their action, is necessary for the origination and establishment of an axial gradient in protoplasm. In other words, the gradients must arise through the differential action of environmental factors which affect primarily the rate of general protoplasmic activity.

Origin of Axiate Pattern in Plants and Simpler Animals.—As regards the plants, Winkler ('00b) and Kniep ('07) showed that in certain algæ, *e.g.*, various species of Fucaceæ, differential illumination of the two sides of the egg determines the polarity of the plant developing from the egg, though in the absence of light polarity appears and germination occurs, but more slowly. Stahl ('85) showed that in the spore of *Equisetum* polarity is determined in the same way. Winkler ('00a) also showed that polarity in *Bryopsis* could be determined by light. In various

bilaterally symmetrical plants, such as liverworts, light determines the dorsiventrality, and some algæ develop a radially symmetrical thallus when the illumination is equal on all sides of the polar axis and are bilateral when the illumination is from one side. In the spermatophytes conditions which determine egg polarity are undoubtedly intraorganismic. The embryo sac shows a definite polarity with respect to surrounding parts, and the ovum is attached to one end of the sac. The unattached end of the ovum becomes the apical, the attached end the basal pole of the embryo. What particular factors are concerned in this case is not known.

In the simpler animals we see new polarities arise at cut ends of pieces, *e.g.*, the development of new apical regions and axes from the aboral ends of pieces in various hydroids, the development of heads from posterior cut surfaces in *Planaria*. In such cases the new axis is always represented by a new gradient, and the relation between the new axis and the occurrence of differential exposure is obvious, though whether the wound stimulus, oxygen supply, or some other factor is chiefly concerned in determining the new gradient is not known. Loeb ('92) has maintained that new polarities are determined by gravity in the regulatory development of the hydroid *Antennularia antennina*, but Morgan ('01) and Stevens ('02, '10), while not disputing Loeb's results, showed that other factors besides gravity were concerned in determining polarity.

In the development of sponges from dissociated tissue cells described by H. V. Wilson ('07, '11) the polarity of the new individual is determined by some sort of differential between free and attached surfaces of the cell mass, the osculum developing on the free surface. Similarly in the experiments on obliteration of a preëxisting polarity and the establishment of a new polar axis in hydroids (Child, '15c, pp. 142-146) the apical region of the new axis arises in the region of greatest exposure to the environment, but the particular factors chiefly concerned have not been determined. In both these cases the sponge and the hydroid, the difference in oxygen supply between the exposed surface of the cell mass and the surface in contact suggests a probable factor in determining the new axis.

In the actinian *Harenactis* the localization of a region of more rapid and more extensive growth by injury is sufficient under certain conditions to determine the position and development of new polarities (Child, '09, '10b, '15c, Figs. 79-83). The axial gradation results in this case from the fact that the activity of the cells is greatest in the middle region of such an area and decreases toward its borders. In this connection a recent statement of Harrison's concerning the limb-rudiment of *Amblystoma* is of interest. Harrison says: "The limb rudiment may be thus regarded, not as a definite circumscribed area like a stone in a mosaic, but as a center of differentiation in which the intensity of the process diminishes as the distance from the center increases, until it passes away into an indifferent region. Many other systems, such as the nose, ear, hypophysis, gills, seem to have the same indefinite boundaries which may even overlap one another" (Harrison, '18, p. 456). In other words, Harrison conceives these primordia as gradients in activity in a more or less specialized cellular region of the embryo. Such gradients differ from the general axial gradients of the body only in that they are determined in some way, presumably by intraorganismic correlative conditions in specialized body regions, and are concerned with particular organ complexes instead of with the body as a whole. In still other cases new polarities are apparently determined and localized by slight differences in activity between different cells of a mass. Such differences determine the more or less definite localization of a region of growth in which the activity decreases toward the periphery and as growth progresses an axis arises. Determination of new polarities in this way apparently occurs in many cases when pieces of naked hydroid stems give rise to multiple stolons, each of which represents a new axis and a new gradient. These multiple polarities have been observed by many investigators, and I have been able to produce them experimentally in hydrozoan planulæ by first obliterating the original polarity through differential inhibition. In the origin of adventitious buds from the epidermal cells of the *Begonia* leaf similar local growth areas with gradients in activity from center toward periphery and from the surface inward are the first indications of the new plant axes (Regel, '76, Child,

'15c, Figs. 38, 39). The localization of the new axis in such cases appears to be largely a matter of slight fortuitous differences in activity in different cells or cell groups in consequence of which certain cells or groups react more rapidly than others to the experimental conditions.

The Animal Egg.—As regards the animal egg, the evidence is very incomplete, but indicates that in at least many forms polarity is determined during the growth period of each egg by differential exposure. In various hydromedusæ for example the growing oögonia constitute a columnar epithelium, one end of each cell being separated from the exterior only by a layer of very thin flattened cells, while the opposite end is attached and adjoins the radial canal. When portions of the medusa ovaries are slightly teased to separate the eggs it is found that the free end of the egg, the end nearest the exterior, represents the high region, the attached end the low region of a gradient in susceptibility and permanganate reduction. A similar gradient appears in developmental stages and while the absence of good landmarks makes it impossible to demonstrate that the later gradient is identical with the earlier, there can be little doubt that it is. In the sea urchin the oögonium is attached to the wall of the ovary at one small region of its circumference and here also, as Boveri has shown, the free pole becomes the apical pole and represents the high end of a gradient. In these cases it is not the pole through which nutrition enters, but the unattached pole which in the medusa is more exposed to external factors and in the sea urchin to the fluids of the ovary which becomes the apical pole. It seems probable that a differential in oxygen supply and perhaps also in CO_2 concentration are chiefly concerned in determining the polarity and the gradient which represents it in these cases. Even in the worm *Sternaspis*, in which a peduncle containing a vascular loop develops in connection with each growing oögonium, the attached pole of the egg, into which the vascular loop enters becomes the basal pole.

In the eggs of the higher animals where oxygen as well as nutritive substances reach the egg chiefly or wholly through the blood, the polarity may apparently be determined by relation to the blood supply. Bellamy ('19) has shown that in the frog's egg

polarity apparently develops in definite relation to the vascular supply, the apical pigmented pole arising on the arterial, the basal unpigmented pole on the venous side. In these cases the oxygen supply or the conditions determining rate of respiratory exchange are apparently the chief factor in determining polarity, the region of most rapid exchange becoming the apical pole.

Symmetry in animals, like polarity is indicated by gradations in physiological condition, and although a particular kind of protoplasm may give rise normally to a radial or a bilateral animal, experiment shows, that for certain forms at least, the normal symmetry of pattern is not inherent and unchangeable. In the radial anemone *Harenactis*, for example, bilateral tentacle groupings may arise under certain experimental conditions (Child, '09) and it is possible through differential inhibition to obliterate bilaterality and produce radial larval forms in the sea urchin (Child, '16*d*) and also in the starfish (unpublished). Moreover, in pieces of *Planaria*, under experimental conditions which practically obliterate the polar gradient the symmetry gradient may become the polar axis of the new individual (Child, 15*c*, pp. 163-165).

A characteristic feature of radial symmetry is the repetition of parts, usually axiate and often bilateral, in pattern about a center. Such repetition must be largely a matter of the space relations of specialized growth centers. Each growth center involves or dominates a certain area, and only at a certain distance from it can another similar center arise. Thus the number of such growth centers arising on a given circumference depends on the area dominated by each center which varies with physiological condition and on the size of the circumference and can be altered experimentally in many cases. Moreover, as growth in size of the circumference occurs and the distance between the repetitive parts increases we often find additional new parts arising, *e.g.*, mesenteries and tentacles in many actinians, etc. Such processes are physiologically similar to many forms of agamic reproduction, being essentially reproductions of specialized parts instead of new wholes, resulting from physiological isolation (Child, '15*c*, Chaps. IV., V.).

The Problem of Symmetry.—Bilaterality is inherited through

many processes of agamic reproduction, *e.g.*, in flatworms and annelids and may of course also be inherited in many eggs. On the other hand, it may conceivably be determined in some eggs by ovarian conditions, by conditions connected with maturation or with fertilization, or perhaps even by conditions arising later in development. Moreover, many bilateral forms develop characteristic asymmetries during the course of development, *e.g.*, the asymmetry of gastropod mollusks and the visceral asymmetry of vertebrates, or a well-developed bilateral symmetry may give rise in metamorphosis to a radial-bilateral pattern of very different kind, as in certain echinoderm groups. Our knowledge concerning the physiological aspects of the origin of symmetry in animals is still very fragmentary, but the earliest indications of the presence of a particular symmetry pattern are gradients in physiological condition, which, so far as the evidence goes, are similar to the polar gradients, and in plants we see the different symmetry patterns arising through differential exposure to the action of external factors. In the light of all the facts we are justified in concluding that even though a particular symmetry pattern may persist through reproduction, *i.e.*, be inherited in a particular case, symmetry like polarity must in the final analysis arise through differential exposure to the action of external factors. On the other hand, even if we grant that the differential exposure of the egg of the medusa, the sea urchin, *Sternaspis*, etc. (see p. 173), determines the polarity, and that symmetry may also be determined by relation to environment, it is evident that, except in some of the simpler organisms, the differential exposure of the egg is not fortuitous, but is determined by the hereditary mechanism of the organism. The epithelial arrangement of eggs in the medusa gonad, the position of the sea urchin egg in the ovary, the development of the peduncle and the vascular loop in *Sternaspis*, and the circulatory pattern in the chorion of the frog's egg are all features of the hereditary mechanism of the organism concerned. Even in such cases then, as well as in cases where the axiate pattern persists through reproduction, the hereditary mechanism is concerned in the origin of the axiate pattern of the new individual. Moreover, even in the case of *Fucus* where the determination of polarity by the

direction of incident light is apparently wholly fortuitous, the hereditary mechanism, as expressed in the constitution and pattern of the egg protoplasm, determines the occurrence and the nature of the reaction to incident light. In other words the hereditary constitution of the *Fucus* egg as well as the direction of incident light is a factor in the determination of the polarity. There is, in short, no conflict between this physiological conception of the origin of organismic or of axiate pattern and conceptions of heredity. The origin and development of organismic pattern in nature is simply the realization of certain hereditary potentialities of a particular protoplasm in a particular environmental complex, which may itself be determined in large measure or wholly by the hereditary mechanism of the protoplasm concerned.

Surface-interior Pattern and Axiate Pattern.—In the light of the conclusions reached concerning the nature and origin of axiate pattern, the question of cell pattern, touched upon above (pp. 150–151) requires some further consideration. It was suggested that the cell is primarily a surface-interior pattern resulting from exposure of the surface of a mass of protoplasm to the action of external factors. Such an exposure is a differential exposure as regards surface and interior. Both the respiratory exchange and excitation can occur only through the surface, therefore differences must arise between surface and interior, and a more or less definite gradient in such conditions from the surface inward must result. As different organs are localized at different levels of an axial gradient so the localization and differentiation of the nucleus in the first instance may have resulted from the conditions in the interior of the protoplasmic mass. In fact, it is difficult to see how the nucleus as a definite organ could have arisen otherwise. The differences between nucleus and cytoplasm as regards acidity and electric potential as well as the behavior of nuclei in such specialized cells as spermatozoa, where cytoplasm is practically absent, all suggest that the nucleus is fundamentally an internal cell organ, and if the origin of cell pattern has any relation to environmental factors, the differentiation of the nucleus must have been determined originally by conditions in the interior of a protoplasmic mass. The fact that the

nucleus persists from one cell generation to another means merely that the pattern once established is persistent or inherited, although it is difficult to determine to what extent the persistence of the surface-interior conditions is concerned in the persistence of the pattern.

Viewed from this standpoint, cell pattern originates in the differential between surface and interior in general, and axiate pattern in differential between different parts of the surface of the protoplasmic or cell mass concerned. As regards the axiate pattern, the evidence indicates that the differential is primarily quantitative and involves differences in the rate or degree of fundamental protoplasmic activity, but as regards cell pattern we have at present no means of determining whether the differential was primarily quantitative, though various lines of evidence point in that direction.

The presence of an axiate pattern does not imply the disappearance of a general surface-interior pattern, either in the cell or the multicellular organism. All organisms show some kind of surface-interior pattern at least in the superficial regions of the body, and all the facts indicate that in the final analysis such pattern arises through exposure of the surface. The passage of cells to the interior of the embryo by gastrulation is of course a feature of axiate pattern, but conditions in the interior are undoubtedly factors in determining the further differentiation of such cells into the organs of entoderm and mesoderm. Only in some of the simpler animals does the general surface-interior differentiation arise *in situ*. Formation of entoderm by delamination in all cells of a blastula for example appears to be a case in point, and in the protozoa definite structural differentiation is limited to the ectoplasm. But in the ectoderm of multicellular animals, for example, we find numerous evidences of surface-interior pattern ranging from the basal muscular extensions of ectoderm cells in *Hydra* to various complicated sensory structures and the early differentiation of the neural tube in the higher animals, and the relation of such features of pattern to exposure of the surface appears obvious. Here, however, as in the case of the axiate pattern, the surface-interior relation represents merely the physiological conditions under which the potentialities of the

hereditary mechanism of the protoplasm are realized. The fact that the embryo possesses a surface determines certain relations in the protoplasm to this surface and the specific constitution of the protoplasm determines the kind of reaction which occurs, the sort of specialization which develops. If an axiate pattern is also present, physiological conditions are provided for differences in reaction in relation to the surface at different regions or levels of the body. This of course does not mean that exposure to a special external stimulus is necessary for the development of a particular superficial organ. Light, for example, is not necessary for the differentiation of a photoreceptor. The surface-interior relation merely determines that the physiological conditions under which the hereditary potentialities of a given protoplasm to produce a photoreceptor are realized are conditions which arise in development at or near the surface of the developing organism.

The Gradients in Relation to Excitation and Transmission.—The differential exposure of the cell or cell mass to the action of environmental factors is only the first step in the establishment of the gradient. Admitting that this differential exposure determines a higher rate of activity in some region, we may expect, since living protoplasm is irritable, and since increased activity in one region serves to some extent to excite adjoining regions, that transmission from the region of increased activity will occur. In the absence of highly specialized conducting paths we find that protoplasmic excitation apparently undergoes a decrement in intensity or effectiveness with increasing distance from the point of origin, so that an excitation gradient results. Discussion of the evidence bearing on this point is impossible in this paper, but many facts indicate that the physiological relation resulting from the differential exposure of a cell or cell mass is primarily a relation of excitation and transmission and that the resulting gradient is essentially the fixation or establishment of an excitation-transmission gradient in the protoplasm through the modification of the protoplasmic substratum by the persistence or repetition of the differential exposure. From this viewpoint the gradient represents the most primitive sort of excitation-transmission relation and its effects upon protoplasm.

In a series of recent papers, R. S. Lillie has advanced a

theory of excitation and transmission¹ in terms of the plasma membrane, its electrical depolarization in excitation with increase in permeability and resulting electric current. The membrane changes in excitation are conceived by Lillie as involving a chemical reaction, probably oxidative, and the electric current resulting from the depolarization at any point is regarded as the agent of transmission, since it tends to produce depolarization at other points within a certain range and so induces excitation at those points.

Lillie's theory seems to me to account very satisfactorily for a wide range of observed facts, particularly in the more highly specialized excitations and transmissions, but the question may be raised whether the depolarization of the membrane is always the primary factor in excitation or the chief source of the electric current. A region of rapid oxidation gives rise to an electric current similar to that which Lillie regards as arising from the depolarization of the membrane. Doubtless in protoplasm membrane changes and oxidations are not independent, but while the highly specialized process in the nerve may be very largely or wholly a membrane process, it seems probable that in the primitive excitation processes the oxidations play a more important, perhaps the chief part, and that the excitation is not necessarily limited to the plasma membrane. In his later papers Lillie apparently recognizes and admits this probability.

As Lillie points out, the strength of the current produced at a point of excitation decreases with increasing distance from that point, because of resistance, and it can act as a stimulus only within a certain distance. In other words, this agent of transmission undergoes a decrement or represents a gradient from the point of original excitation, and except in highly specialized tissues which react according to the "all or none" law, the current must determine decreasing degrees or amounts of change and so of excitation at increasing distances from the point of original excitation. Even though these new excitations give rise to sufficient current to excite points beyond the range of the original current the transmission process will show a decrement and

¹ R. S. Lillie, '09a, b, c, '11, '13, '14, '15, '16a, b, '17, '18, '19; R. S. Lillie and E. N. Johnston. '10.

finally cease. Briefly, this means that the primitive excitation-transmission relation in protoplasm must be a gradient with decrement from the region of most intense activity.

We are accustomed to say that excitation is completely reversible, and as regards the nerve fiber it may be true, but certainly memory and the possibility of learning demonstrate that at least in certain parts of the nervous system reversibility is not complete. Similarly in muscle the contraction is reversible, but repeated excitation within limits brings about growth, *i.e.*, functional hypertrophy. The fully developed organism has reached or approaches a condition of dynamic equilibrium in which most changes except the progressive changes of age are relatively reversible. Most of the less readily reversible changes have already occurred during development. But embryonic protoplasm stands at the beginning of the developmental process of equilibration, and while the momentary and more superficial excitations are undoubtedly largely or wholly reversible, conditions which persist for any considerable length of time must produce modification in the protoplasmic substratum and this may be more or less permanent, according to the nature of the protoplasm. According to this conception, then, the physiological gradients arise as primitive excitation-transmission relations in protoplasm, and if the differential exposure persists long enough they become more or less persistent and constitute physiological axes or radii.

Objection to this conclusion may be raised on the ground that oxygen supply or rate of removal of CO_2 which have been regarded as factors concerned in determining gradients are not properly speaking "stimuli" but rather specific material relations between protoplasm and environment. As a matter of fact, however, we know that sufficient differences in oxygen supply or in rate of removal of CO_2 do determine differences in the rate of the respiratory activities of protoplasm, and the respiratory activities are apparently essential factors in excitation, particularly in the more primitive excitation processes. As already suggested, many facts indicate that excitation in highly specialized protoplasms with special structural mechanisms may differ more or less widely from the process as it occurs in protoplasm in general, but we are concerned here rather with the process in its

most general terms than with such specialized processes. Excitation in the broad sense is apparently an increase in the rate of living or at least of that aspect of life which concerns energy liberation, and the respiratory relations to environment are factors in determining that rate. If a region of higher rate of respiratory activity is determined in a cell or cell mass by differential exposure to oxygen or to removal of CO_2 that region is in a state of excitation as compared with other regions and the relations between it and such other, regions are primarily those which exist in general between excited and unexcited regions. Oxygen supply and CO_2 are then not only specific material factors in the relation between protoplasm and environment, but they are or may be quantitative factors in this relation.

From the first moment when differences at different levels of the gradient begin to arise, the possibility of chemical or transportative correlation arises, and as the progressive complication occurs and different substances appear in the protoplasm at different levels, these possibilities become always increasingly varied complex and specific. On the other hand, it is evident that definite and orderly chemical or transportative correlation as between protoplasmic regions rather than protoplasmic constituents cannot occur until definite and orderly regional differences are present. The gradient is merely the first step in the development and evolution of axiate pattern, and the progressive complications and alterations of the relations and changes initiated by its appearance are terminated in the individual only by agamic reproduction or some other process involving dedifferentiation and reorganization, or by death, and as regards its evolutionary termination, even speculation is idle.

CONCLUSION.

It is perhaps necessary to point out that the conception with which this paper is concerned, the conception of axiate pattern as primarily a quantitative gradation in physiological condition, is nothing more than an attempt to interpret certain aspects of the physiology of development. The axial gradients do not create anything, they are not the "cause" of growth or differentiation in the organism, they do not determine what organs shall develop

in a particular protoplasm. Granting their existence and the significance which I have assigned to them they represent merely certain physiological conditions, under which the hereditary mechanism of a protoplasm gives rise to the order or pattern which we call axiate or axiate-symmetrical.

The egg in most cases requires fertilization or the action of some other factor external to it to initiate development, but the specific hereditary constitution of the egg protoplasm with its potentialities of development is present, whether fertilization or initiation of development by other means occurs or not. Physiologically speaking the spermatozoön or some other factor external to the egg merely sets the mechanism in motion or gives it the necessary speed and development proceeds. Similarly the gradient in the egg, whether it persists from earlier cell generations or arises anew in the egg through differential exposure, is merely a physiological condition which determines that the hereditary mechanism shall give rise to a particular order or pattern. Alteration of the gradient relations alters the pattern though the hereditary mechanism remains the same. The gradient is then nothing more than one of the physiological conditions under which the development of axiate organisms occurs, and the surface-interior relation, whether a gradient or not, is merely another even more general physiological condition of organismic development.

The idea of a quantitative gradient in physiological condition as the condition initiating axiate development and of its origin in the final analysis through the action of an external factor does not require or depend upon any particular assumptions or theories concerning inheritance or evolution. Some of the critics of the conception have regarded it as Lamarckian, but it is not, though of course it might be used if desired in a Lamarckian way. Strictly speaking, it has nothing directly to do with either inheritance or evolution, except in so far as it maintains that axiation or polarity and symmetry are not inherent properties or characteristics of protoplasm. It is fundamentally a physiological conception formulated on the basis of many different lines of observational and experimental evidence, and while its formulation in the present state of our knowledge is necessarily

incomplete and perhaps vague, I believe a fair consideration of the evidence now available justifies the conclusions advanced.

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